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(54) Title: TREATMENT OF TRICHOMONAL INFECTIONS WITH DITIOCARB OR DISULFIRAM

(57) Abstract

This invention relates to the use of the compound disulfiram or copper complexes thereof or its active metabolites or copper complexes thereof for the manufacture of a medicament for the treatment of a subject suffering from an infection caused by a pathogen of the order Trichomonadida.

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TREATMENT OF TRICHOMONAL INFECTIONS WITH DITIOCARB OR DISULFIRAM

Field of the Invention

This invention relates to the control and treatment of diseases caused by the protozoan pathogens of the order *Trichomonadida*, especially *Trichomonas vaginalis* and *Tritrichomonas foetus*.

Background to the Invention

Trichomoniasis is a common cause of vaginitis in women and of urethritis in men. It is caused by the protozoan parasite *Trichomonas vaginalis*. The condition is localised and considered to be sexually transmitted in the majority of patients. Trichomoniasis is highly prevalent in Western countries and is one of the most common sexually transmitted diseases. Pathogens belonging to the order *Trichomonadida* (hereinafter referred to as trichomonads) are flagellate protozoa with 3 - 5 anterior flagella, an axostyle and an undulating membrane. They can be divided into several genera including, *Trichomonas*, *Pentatrichomonas*, *Tritrichomonas* and the related genera *Histomonas* and *Dientamoeba*, which may be non-flagellated. In humans, the pathogens are *T. vaginalis* and *D. fragilis*. In animals, pathogens include *Tri. foetus* in bovines, *T. gallinae* which infects pigeons and *Histomonas meleagridis* which causes blackhead disease in turkeys. This invention is of interest to all genera but is particularly applicable for use in humans, especially against *T. vaginalis* and in bovines, especially against *Tri. foetus*.

It was found in 1959 that metronidazole was effective against *T. vaginalis* (Corsar, C. and Julou, L. 1959, Ann. Instit. Pasteur, 96, 238-241) and this still remains the first choice of drug in the treatment of trichomoniasis. Metronidazole resistance in trichomoniasis was noticed in 1978 (Meingasser. J. G., and Thurner, J. 1978, The Lancet 2 738) and has since been reported with increased frequency. However, most cases of trichomoniasis can still be treated with metronidazole using higher dose regimes. True resistance to metronidazole is rare, treatment failure being in most cases due to re-infection, or non-compliance, and, in these cases, niridazole is more effective than metronidazole in treating these highly resistant strains (Yarlett, N., et al, 1987, Parasitology, 94, 93-99). Unfortunately, metronidazole has toxic side effects. Thus at

present, for some patients with highly resistant strains of *T. vaginalis*, trichomoniasis remains an untreatable disease. It is desirable to develop an effective treatment for such patients.

Tritrichomonas foetus infection of cows can cause early embryonic death, abortion, pyometra and infertility of dairy and beef cows. Bulls are also infected with this parasite but show no clinical signs. Current prevention of trichomoniasis in bovines requires the maintenance of a closed herd and a rigorous programme of breeding management. Limited therapeutic treatment of trichomoniasis in bulls is at present available and infection in cows can only be prevented or attenuated by vaccination with a product containing killed *Tri. foetus* organisms. It is clearly desirable to develop a treatment for bulls which can also be used in cows.

The compound bis(diethylthiocarbamoyl)disulphide [hereinafter referred to as disulfiram often with the spelling disulphiram] is licensed for use in the management of alcoholism. The compound is known under the name "Antabuse" and is tolerated in high dosages. Treatment is based on the extremely unpleasant but generally self-limiting systemic effects which occur when a patient receiving the drug ingests alcohol. Disulfiram has also shown some anti-parasitic activity in in vitro studies of *Trypanosoma brucei* (Cross, G.A.M. et al., 1975, Parasitology, 71, 311-326) and *Plasmodium falciparum* (Scheibe, A., et al, 1979, PNAS USA 76 5303-5307) but, it has never been tested as an anti-trichomonal compound.

Summary of the Invention

It has now been surprisingly found that the compound disulfiram and active metabolites thereof are effective in the treatment of trichomoniasis, especially against the parasites *Trichomonas vaginalis* and *Tritrichomonas foetus* and more especially against metronidazole-resistant strains of these organisms.

The present invention comprises the use of the compound disulfiram or an active metabolite thereof for the control of infections caused by these organisms. The compound(s) may be used for the manufacture of a medicament for the treatment of a subject suffering from an infection caused by a pathogen belonging to the order

Trichomonadida, especially those of the genera *Trichomonas* and *Tritrichomonas*. In addition to *T. Vaginalis* and *Tri. foetus* other trichomonads of veterinary importance are *T. Gallinae*, *Histomonas meleagridis* and *Dientamoeba fragilis*.

Disulfiram is known to be metabolised *in vivo*. Metabolites of disulfiram are, first, the reduced product diethylthiocarbamate (Ditiocarb) and then certain oxidation products including the compounds S-methyl N,N-diethylthiocarbamate sulphoxide, and S-methyl N,N-diethylthiocarbamate (Hart *et al.*, Alcoholism, Clinical & Experimental Research 18, 340-345, 1994) and S-methyl N,N-diethylthiocarbamate sulphone (Mays *et al.* Biochemical Pharmacology, 49, 693-700, 1995).

Meshnick S.R. *et. al.*, Biochem. Pharmacol., 40, 213-216, 1990 report that the antimalarial activity of disulfiram is potentiated by complexing it with copper. There is evidence that the same effect occurs with anti-trichomonal activity. Thus, the invention also comprises the use of a copper complex of disulfiram or a copper complex of an active metabolite of disulfiram for the control of infections caused by a trichomonas species.

The use of the invention may also be described as a method of treating a subject suffering from an infection caused by a pathogen of the order Trichomonadida which comprises administering to the subject in need thereof, a therapeutically effective amount of the compound disulfiram or an active metabolite of disulfiram.

Testing of the compounds against pathogenic organisms was carried out as described below.

Test Procedure

Strains of *T. vaginalis*

Four isolates of *T. vaginalis* were used. These were the metronidazole-sensitive strain 1910 described in Ackers *et al.*, Br. J. Vener. Dis. 1975, 51, 319-323, the resistant strains IR-78 (Meingassner *et al.* 1979, Antimicrob. Agents Chemother. 15, 254-257) "Fall River" (Muller *et al* 1980, Amer. J. Obstet. Gynaecol. 138 808-812) and CDC-085 (Lossick, J.G. *et. al.*, J. Inf. Dis. 153, 948-955, 1988).

Strains of *Tri. foetus*

Tri. foetus D-1 strain was as described in BonDurant *et. al.*, Infect. Immun. 61, 1385-1394, 1993.

Cultivation of *Trichomonas* strains

T. vaginalis strains 1910, 'Fall River', IR-78 and CDC-085 and *Tri. foetus* Strain D-1 were cultured in TPS-1 medium (Diamond *et al.*, 1978, Trans. R. Soc. Med. Hyg. 72, 231-232). Stock cultures were maintained at 37°C and subcultured daily.

Drug Susceptibility Assay

Materials

Metronidazole (Zadstat) injection-solution 5 mg/ml. Disulfiram (tetraethylthiuram disulphide) was obtained from Sigma. A stock solution was prepared by dissolving the drug in dimethyl sulfoxide (DMSO) to a concentration of 8 mg/ml.

Method

A modification of the multiwell method described by Meingassner *et. al.* (1978, Antimicrob. Agents Chemother. 13, 1-3) was used. Flasks with 24 hour old cultures were chilled in an ice-water bath for 10 minutes, shaken on rocking table to detach adhered cells, centrifuged for 5 minutes (1000 rpm), and resuspended in fresh TPS-1 prepared without cysteine and ascorbic acid (further indicated as TPS-1*). After counting in an 'improved Neubauer' haemocytometer the density was adjusted to 1,000,000 organisms per ml.

The stock concentrations of the drugs were diluted with TPS-1* medium to 2 times the highest concentration 100 µl was pipetted in the first column of a 96-well microtiterplate, of which every well was filled with 100 µl TPS-1* medium. Two-fold dilutions were obtained by serial dilution with a multichannel Titertek pipet (Flow laboratories). 100 µl of the parasite suspension was added to the wells containing the 100 µl of TPS-1* diluted drug. Eventually each well contained a total of 100,000 parasites in 200 µl. (Rows with drug dilutions in 2 fold).

The microtiterplates were covered with a sterile lid and incubated at 37°C in an incubator for aerobic testing, and in an aerobic cabinet (Mark II, filled with an anaerobic gas mixture: 10% H_2, 10% CO_2 and 80% N_2) or Oxoid sachet in a closed jar for anaerobic testing.

The wells of the microtiterplate were examined after 24 hours under an inverted phase-contrast microscope (400 x). Viability was assessed by motility of the organism and flagellae. Minimal Lethal Concentrations (MLC-24) was defined as the lowest concentration value where no motility of any of the organisms was observed after 24 hours of drug incubation. To confirm the observed values, subsequent viability testing was carried out by subculturing all remaining organisms after a washing in PBS and incubation in fresh TPS-1 medium for 2 days.

RESULTS

The presented data were obtained from an experiment in a series of experiments with similar results and are shown in Table 1 below. Susceptibility results varied with the amount of reducing agents added to the test-medium (Cysteine and ascorbic acid). Reproducible results (variation of a single dilution) were obtained with a standardized medium. MLC-24 values were confirmed after subculturing all organisms after drug incubation for 48 hours. In some cases motile organisms did not survive, and MLC values were (one dilution) lower. Data shown was based on motility after 24 hours.

TABLE 1

(MLC = Minimal Lethal Concentration)

Species	Known Metronidazole Sensitivity	MLC FOR Metronidazole µg/ml		MLC FOR Disulfiram µg/ml	
		aerobic	anaerobic	aerobic	anaerobic
T. vaginalis					
1910	Sensitive	3	0.75	0.2	0.1
Fr	Intermediate resistant	25	1.56	0.4	0.2
IR-78	Intermediate resistant	25	0.4	0.1	0.1
CDC085	Highly resistant	100	3.13	0.2	0.2
Tri .foetus					
D-1	Sensitive	3.13	1.56	0.1	0.2

The minimal lethal concentration (MLC) values for metronidazole under aerobic conditions for the known resistant strains 'IR-78', 'Fall River' and 'CDC-085' are 4 - 30 times higher than for the sensitive *T. vaginalis* '1910' and *Tri. foetus* 'D-1'. Under anaerobic conditions MLC values are lower, and in the same range for sensitive and resistant strains.

For disulfiram, MLC-values in µg/ml are lower than for metronidazole. No clear difference was found between the sensitive and resistant strains, nor between aerobic and anaerobic test conditions.

In metronidazole resistant strains the susceptibility to disulfiram under aerobic conditions is 100-1000 times higher than to metronidazole.

The endpoint of the assay with disulfiram was very clear and usually reached in 12 hours of drug incubation. This was different from metronidazole in which a concentration dependent gradient was observed. Also the toxic effect of metronidazole was more extended over time, as reflected in different MLC-values for 24 and 48 hour incubation (data not shown).

CONCLUSIONS

Trichomonas vaginalis and *Tritrichomonas foetus* showed a high susceptibility to disulfiram *in vitro* compared with metronidazole. Under aerobic conditions, *in vitro*, when metronidazole resistance becomes apparent (in the metronidazole resistant strains) disulfiram appears equally effective against metronidazole sensitive strains as against metronidazole resistant strains of *T. vaginalis*. This resistance under aerobic conditions, correlating with resistance *in vivo*, is a long known phenomenon. The oxygen dependent effect of metronidazole was not observed in disulfiram under identical conditions of the same experiment.

Administration of Disulfiram, Ditiocarb and other compounds

The MLC values of Disulfiram and Ditiocarb are of the same order as those of metronidazole and other 5-nitroimidazoles. Administration of compounds in accordance with this invention may be carried out using dosages similar to those used for metronidazole in humans and ipronidazole and dimetridazole in cattle.

The compounds may be administered in any pharmaceutically acceptable form, but are preferably orally or topically administered. Disulfiram or its active metabolite or copper complexes thereof may be formulated with a physiologically acceptable diluent or carrier for use as a pharmaceutical for human or veterinary use by a variety of methods. For instance, the compound may be applied as a composition incorporating a liquid diluent or carrier, for example an aqueous or oily solution, suspension or emulsion, which may often be employed in injectable form for parenteral administration and therefore may conveniently be sterile and pyrogen free. Oral administration may also be used. Although compositions for oral use may incorporate a liquid diluent or carrier, it is preferred to use a solid, for example a conventional solid carrier material such as starch, lactose, dextrin or magnesium stearate. Such solid compositions may conveniently be of a formed type, for example as tablets, capsules (including spansules), etc.

Other methods of administration than by injection or orally may also be used in both human and veterinary contexts, for example, topical formulations, suppositories or pessaries. Another method is by buccal or nasal administration, for example, using lozenges, nose drops or an aerosol spray, or alternatively drops for administration into the eye which may conveniently contain a sterile liquid diluent or carrier.

The compound disulfiram or active metabolites thereof are preferably formulated in unit dosage form. A suitable dosage will depend on the subject being treated. By way of guidance for humans, when orally administered, the dosage of disulfiram will be in the range 400 mg to 1 g per kg of body weight daily.

For human use against *T.vaginalis*, local application is recommended in the form of a vaginal pessary.

For the treatment of *Tritrichomonas foetus* infection in bulls it is presently preferred to deliver the compound in an emulsion, foam or ointments vehicle. The vehicle should be capable of delivering an effective dosage to the penile crypts of the bull and have sufficient viscosity to adhere to the penile and preputial cavity of the animal. It should be fluid enough to be deliverable at low pressure. An emulsion, an expanding foam and/or an ointment will have the desired properties.

While either oil-in-water or water-in-oil emulsions may be used, the latter are preferred due to their higher viscosity. Oil-in-water emulsions may be prepared from mineral, vegetable or animal oils, stabilized as droplets in the water phase by emulsifiers such as sodium stearate, triethanolamine stearate, and sodium stearylsulphate, in concentrations up to 1%. The emulsifier may also comprise one of the polysorbates, which are polyoxyethylene fatty acid esters. In particular, the emulsifier may advantageously comprise polyoxyethylene (ZO) sorbitan monooleate more commonly known as polysorbate 80.

Water-in-oil emulsions may be prepared from mineral, vegetable or animal oils, where the water phase is stabilized as droplets in the oil phase by emulsifiers such as lecithin, stearyl alcohols, cholesterol and sorbitan. in concentrations up to 5%. The emulsifier may advantageously also comprise one of the fatty acid partial esters of sorbital anhydrides. such as sorbitan monooleate.

Either aqueous or non-aqueous foams may be used for local delivery. Non-aqueous foams are typically prepared from polyalcohols such as propylene glycol or glycerol and stabilized with emulsifiers such as any of the emulsifying waxes available from Croda. Inc. under the trademark Polawax.RTM. or any of the polyoxyethylene ethers of higher aliphatic alcohols available from ICI Americas. Inc. under the trademark Brijo RTM. Isobutane or other polyalcohol-soluble gases can be used as expellers. Aqueous foams may be prepared from a mixture of polyalcohols (propylene glycol. glycerol. etc.) and water, and stabilized with emulsifiers such as the "Softigen" emulsifiers available from Hals AG, Polawax.RTM. and/or "Montawax" .

According to yet another embodiment, the delivery vehicle may comprise an ointment, prepared from mixtures of oils and waxes, and quantitatively balanced in order to obtain the desired physical, chemical and mechanical properties.

Delivery of formulations for the treatment of *Tri.foetus* infection may be achieved with a propellant such as compressed air or an inert gas. The compressed air or gas will deliver the formulation through an applicator inserted into the animal's preputial cavity. Alternatively, a syringe may be used.

For most bovine trichomoniasis infections. a delivered volume ranging between about 50-100 ml should ensure treatment of all surface areas. Preferably, during treatment, the applicator should be rotated while within the preputial cavity.

In the treatment of *Tritrichomonas gallinae* infections in gallinaceous birds (i.e., birds having a crop). it is presently preferred to administer the compound in the bird's food in such a manner that it is retained in the crop. A preferred dosage will generally range from between about 100 to about 500 grams of compound per ton of bird feed.

FURTHER TEST RESULTS

The results obtained to date are given in full in Tables 2-5.

The most consistent correlation between *in vitro* MLCs and clinical outcome is found when metronidazole is tested under aerobic conditions without cysteine or ascorbate in the medium. Direct measurement has shown that the environment in the human vagina is microaerophilic and thus this correlation is logical. Under these conditions Disulfiram (DIS) and Ditiocarb (DDC) are highly active against a number of strains of *T.vaginalis* and *Tri.foetus*, (Tables 2 and 3) including strains of both resistant to the standard drug metronidazole (ATCC 50142, 50143 and 50151, 50152, respectively):

Oxidation of disulfiram is necessary for activity which is reduced under anaerobic conditions and in the presence of reducing agents (Table 4). Copper potentiates the action of disulfiram against both *T.vaginalis* and *Tri.foetus* (Table 5).

Table 2

Minimum lethal concentration (MLC) values (μg/ml) for *Trichomonas vaginalis* using the medium of Müller *et al.*(*Sexually Transmitted Diseases* **15**:17-24, 1988) without cysteine or ascorbate under aerobic conditions (MET = metronidazole; DIS = disulfiram (disuphiram, bis(diethyldithiocarbamoyl)disulphide), DDC = diethyldithiocarbamate (ditiocarb). No. = number of tests done, each performed in duplicate. Results are means unless divergent results were obtained, in which case the range is given.

Strain	No.	MET	DIS	DDC
1910	4	3.13-6.25	0.025	01-0.2
ATCC 30001	4	0.78-1.56	0.03-0.05	0.4-0.8
ATCC 50138	2	23-25	0.1	0.39
ATCC 50141	1	3.13	0.05	1.56
ATCC 50142	2	50-100	0.05	0.78
ATCC 50143	2	>100	0.05	0.78
ATCC 50144	1	6.25	0.05	0.39

Table 3

Minimum lethal concentration (MLC) values (μg/ml) for *Tritrichomonas foetus* using the medium of Müller *et al.* (*Sexually Transmitted Diseases* **15**:17-24, 1988) without cysteine or ascorbate under aerobic conditions (MET = metronidazole; DIS = disulfiram (disuphiram, bis(diethyldithiocarbamoyl)disulphide), DDC = diethyldithiocarbamate (ditiocarb). No. = number of tests done, each performed in duplicate. Results are means unless divergent results were obtained, in which case the range is given.

Strain	No.	MET	DIS	DDC
ATCC 30003	4	3.1-6.3	0.02	0.1-0.2
ATCC 30166	2	3.1-6.3	0.1-0.2	0.39
ATCC 30231	2	0.8-1.6	0.2-0.4	0.8-1.6
ATCC 30232	3	1.6-6.3	0.05	0.2-0.4
ATCC 30233	3	2-12	0.05-0.1	0.8
ATCC 30924	2	3.1-6.3	0.1	0.2
ATCC 50151	2	50->100	0.1	0.78
ATCC 50152	2	>100	0.1	0.4-0.8

Under anaerobic conditions the presence of cysteine and ascorbic acids (both reducing agents) some strains of both species become resistant to Disulfiram and/or DDC. This suggests that oxidised metabolites of these compounds are the active agents. For example:

Table 4

Minimum lethal concentration (MLC) values (μg/ml) for *Trichomonas vaginalis* using the medium of Müller *et al.* (*Sexually Transmitted Diseases* **15**:17-24, 1988) under anaerobic conditions (MET = metronidazole; DIS = disulfiram (disuphiram, bis(diethyldithiocarbamoyl)disulphide), DDC = diethyldithiocarbamate (ditiocarb). No. = number of tests done, each performed in duplicate. Results are means unless divergent results were obtained, in which case the range is given..

Strain	No.	MET	DIS	DDC
ATCC 30001	4	0.4-0.8	0.4-100	3.13-100
ATCC 50138	2	1.56-2	6.25-7.8	63-100
ATCC 50141	3	0.8-6.3	>100	>100
ATCC 50142	3	3.1-6.3	50->100	50->100
ATCC 50143	2	3.13	100	100
ATCC 50144	2	0.78	50-100	50-100
ATCC 50148	2	1.56	25	50

Table 5

Minimum lethal concentration (MLC) values (μg/ml) from a single experiment performed in duplicate using the medium of Müller *et al.*(*Sexually Transmitted Diseases* **15**:17-24, 1988) without cysteine or ascorbate but in the presence of 1 mole/mole Cu^{++} under aerobic conditions (MET = metronidazole; DIS = disulfiram (disuphiram, bis(diethyldithiocarbamoyl)disulphide), DDC = diethyldithiocarbamate (ditiocarb).

Species & strain	Drug	MET	DIS	DDC	Cu^{++} alone
T.vaginalis	alone	3.13	0.1	0.2	12.5
1910	drug + Cu^{++}	6.25	<0.05	<0.05	-
Tri. foetus	alone	0.39	0.2	3.13	50
ATCC 30232	drug +Cu^{++}	1.56	0.1	0.2	-

CLAIMS

1. The use of a compound selected from the group consisting of disulfiram, ditiocarb, active metabolites of disulfiram, and copper complexes thereof for the manufacture of a medicament for the treatment of an infection caused by a pathogen of the order Trichomonadida.

2. The use according to Claim 1, wherein the subject to be treated is a human.

3. The use according to Claim 2, wherein the pathogen is of the genus *Trichomonas*.

4. The use according to Claim 3, wherein the *Trichomonas* species is *Trichomonas vaginalis*.

5. The use according to Claim 1, wherein the subject to be treated is a bovine.

6. The use according to Claim 5, wherein the pathogen is of the genus *Tritrichomonas*.

7. The use according to Claim 6, wherein the *Tritrichomonas* species is *Tritrichomonas foetus*.

8. The use according to any preceding claim wherein the medicament is in a form suitable for oral administration.

9. The use according to any of Claims 1- 7, wherein the medicament is in a form suitable for topical administration.

10. A method of treating a trichomoniasis infection which comprises administering to a subject in need thereof a therapeutically effective amount of a compound selected from the group consisting of disulfiram, ditiocarb, active metabolites of disulfiram, and copper complexes thereof.

11. A method according to Claim 10, in which the subject is infected with *Trichomonas vaginalis*.

12. A method according to Claim 10, in which the subject is infected with *Tritrichomonas foetus*.

13. A pharmaceutical composition containing a compound selected from the group consisting of disulfiram, ditiocarb, active metabolites of disulfiram, and copper complexes thereof said composition being formulated for topical administration for the treatment of trichomoniasis in humans.

14. A composition according to Claim 13, in the form of a vaginal pessary.

15. A pharmaceutical composition containing a compound selected from the group consisting of disulfiram, ditiocarb, active metabolites of disulfiram, and copper complexes thereof formulated for veterinary application.

16. A composition according to Claim 15, said composition being formulated for treatment of *Tritrichomonas foetus* infection in bovines.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCI/GB 96/01873

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/27 A61K31/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	INVEST. UROL., vol. 18, no. 6, 1981, pages 411-417, XP000609728 J.N. KRIEGER: "Urologic aspects of trichomoniasis." ---	
A	US,A,4 532 122 (WYSOR ET AL.) 30 July 1985 ---	
A	EP,A,0 343 268 (BIOGAL) 29 November 1989 -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Information on patent family members

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